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Title INACTIVE VARIANTS OF THE HUMAN TELOMERASE CATALYTIC SUBUNIT

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INACTIVE VARIANTS OF THE HUMAN TELOMERASE CATALYTIC SUBUNIT

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application 09/128,354, filed August 3, 1998, pending; which is a continuation-in-part of U.S. Patent Application 09/052,864, filed March 31, 1998, now abandoned.

The aforelisted priority applications are hereby incorporated herein by reference in their entirety, as are the following: U.S. Applications 08/851,843; 08/854,050; 08/911,312; 08/912,951; 08/915,503; 08/974,549; and 08/974,584; and International Applications PCT/US97/17618 and PCT/US97/17885, which designate the U.S. and were published on October 1, 1998.

FIELD

The present invention is related to the catalytic protein subunit of human telomerase. The invention provides methods and compositions relating to medicine, molecular biology, chemistry, pharmacology, and medical diagnostic and prognostic technology.

BACKGROUND

The following discussion is intended to introduce the field of the present invention to the reader. The citation of various references in this section should not be construed as an admission of prior invention.

It has long been recognized that complete replication of the ends of eukaryotic chromosomes requires specialized cell components (Watson, 1972, *Nature New Biol.*, 239:197; Olovnikov, 1973, *J. Theor. Biol.*, 41:181). Replication of a linear DNA strand by conventional DNA polymerase requires an RNA primer, and can proceed only 5' to 3'. When the RNA bound at the extreme 5' ends of eukaryotic chromosomal DNA strands is removed, a gap is introduced, leading to a progressive shortening of daughter strands with each round of replication. This shortening of telomeres, the protein-DNA structures physically located on the ends of chromosomes, is thought to account for the phenomenon of cellular senescence or aging of normal human somatic cells *in vitro* and *in vivo*. The maintenance of telomeres is a function of a telomere-specific DNA polymerase known as telomerase. Telomerase is a ribonucleoprotein (RNP) that uses a portion of its RNA moiety as a template for telomeric DNA synthesis (Morin, 1997, *Eur. J. Cancer* 33:750). The length and integrity of telomeres and the telomerase expression status of a cell is thus related to entry of a cell into a senescent stage (*i.e.*, loss of proliferative capacity), or the ability of a cell to escape senescence, *i.e.*, to become immortal.

Consistent with the relationship of telomeres and telomerase to the proliferative capacity of a cell (*i.e.*, the ability of the cell to divide indefinitely), telomerase activity is detected in immortal cell lines and an extraordinarily diverse set of tumor tissues, but is not detected (i.e., was absent or below the assay threshold) in normal somatic cell cultures or normal tissues adjacent to a tumor (see, U.S. Patent Nos. 5,629,154; 5,489,508; 5,648,215; and 5,639,613; see also, Morin, 1989, *Cell* 59: 521; Shay and Bacchetti 1997, *Eur. J. Cancer* 33:787; Kim et al., 1994, *Science* 266:2011; Counter et al., 1992, *EMBO J.* 11:1921; Counter et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91, 2900; Counter et al., 1994, *J. Virol.* 68:3410). Moreover, a correlation between the level of telomerase activity in

a tumor and the likely clinical outcome of the patient has been reported (e.g., U.S. Patent No. 5,639,613, *supra*; Langford et al., 1997, *Hum. Pathol.* 28:416). Thus, human telomerase is an ideal target for diagnosing and treating human diseases relating to cellular proliferation and senescence, such as cancer, or for increasing the proliferative capacity of a cell.

SUMMARY

In one aspect, the invention provides an isolated or recombinant hTRT polypeptide that has telomerase catalytic activity. In one embodiment, the hTRT polypeptide has a deletion of at least 25 residues in the regions corresponding to residues 192-323, 200-323, 192-271, 200-271, 222-240, 415-450, 192-323 and 415-450, or 192-271 and 415-450 of hTRT. In a related embodiment, residues 192-323, 200-323, 192-271, 200-271, 222-240, 415-450, 192-323 and 415-450, or 192-271 and 415-450 of hTRT are deleted. The invention also provides a polynucleotide comprising a nucleotide sequence encoding these hTRT polypeptides. In some embodiments, the polynucleotide includes a promoter sequence operably linked to the nucleotide sequence encoding the hTRT polypeptide.

The invention also provides a method of preparing recombinant telomerase by contacting a recombinant hTRT polypeptide containing a deletion as described *supra* with a telomerase RNA component under conditions such that the recombinant protein and the telomerase RNA component associate to form a telomerase enzyme capable of catalyzing the addition of nucleotides to a telomerase substrate. The hTRT polypeptide may be produced in an *in vitro* expression system and/or may be purified before the contacting step. In some embodiments, the contacting occurs in a cell.

The invention further provides a method for increasing the proliferative capacity of a vertebrate cell by introducing into a cell the recombinant hTRT polynucleotide encoding an hTRT deletion variant described *supra*. In a related embodiment, the invention provides a cell, such as a human cell or other mammalian cell, comprising a nucleotide sequence that encodes the hTRT deletion variant polypeptide. The invention provides such cells that have an increased proliferative capacity relative to a cell that is otherwise identical but does not comprise the recombinant polynucleotide.

In a different aspect of the invention, an isolated or recombinant hTRT polypeptide that has a deletion of amino acid residues 192-450, 560-565, 637-660, 638-660, 748-766, 748-764, or 1055-1071, where the residue numbering is with reference to the hTRT polypeptide having the sequence provided in Figure 1, is provided. In a related aspect, the invention provides an isolated, recombinant, or substantially purified polynucleotide encoding this polypeptide, which in some embodiments includes a promoter sequence operably linked to the nucleotide sequence encoding the hTRT polypeptide.

The invention also provides a method of reducing telomerase activity in a cell by introducing the polynucleotide described *supra* (i.e., having a deletion of deletion of amino acid residues 192-450, 560-565, 637-660, 638-660, 748-766, 748-764, or 1055-1071) into a cell under conditions in which it is expressed.

In a related embodiment, the hTRT polypeptide has one or more mutations other than, or in addition to, a deletion of at least 25 residues in the regions corresponding to residues 192-323, 200-323, 192-271, 200-271, 222-240, 415-450, 192-323 and 415-450, or 192-271 and 415-450 of hTRT.

In an other embodiment, the invention provides &&& METHOD

DRAWINGS

Figure 1 shows the amino acid sequence of a 1132-residue human telomerase reverse transcriptase (hTRT) protein (SEQ. ID NO:2).

Figure 2 shows the nucleotide sequence of a naturally occurring cDNA encoding the hTRT protein (SEQ. ID NO:1).

DETAILED DESCRIPTION

I. Introduction

Telomerase is a ribonucleoprotein complex (RNP) comprising an RNA component and a catalytic protein component. The catalytic protein component of human telomerase, hereinafter referred to as telomerase reverse transcriptase ("hTRT"), has been cloned, and protein, cDNA and genomic sequences determined. See, e.g., Nakamura et al., 1997, *Science* 277:955, and copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549. The sequence of a full-length native hTRT has been deposited in GenBank (Accession No. AF015950), and plasmid and phage vectors having hTRT coding sequences have been deposited with the American Type Culture Collection, Rockville, Maryland (accession numbers 209024, 209016, and 98505). The catalytic subunit protein of human telomerase has also been referred to as "hEST2" (Meyerson et al., 1997, *Cell* 90:785), "hTCS1" (Kilian et al., 1997, *Hum. Mol. Genet.* 6:2011), "TP2" (Harrington et al., 1997, *Genes Dev.* 11:3109), and "hTERT" (e.g., Greider, 1998, *Curr. Biol.* 8:R178-R181). Human TRT is also described in the aforereferenced priority applications and U.S. Patent Application Serial Numbers 08/846,017, 08/844,419, and 08/724,643. The RNA component of human telomerase (hTR) has also been characterized (see U.S. Patent No. 5,583,016). All of the aforementioned applications and publications are incorporated by reference herein in their entirety and for all purposes.

Human TRT is of extraordinary interest and value because, *inter alia*, telomerase activity in human cells and other mammalian cells correlates with cell proliferative capacity, cell immortality, and the development of a neoplastic phenotype. Thus, hTRT polypeptides, including the hTRT variants described herein, and polynucleotides encoding hTRT polypeptides, are used, *inter alia* for conferring a telomerase activity (e.g., telomerase catalytic activity, *infra*) in a telomerase-negative cell such as a cell from a human, a mammal, a vertebrate, or other eukaryote (see, e.g., Bodnar et al., 1998, *Science* 279:349 and copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549). Variants that lack at least one hTRT activity (e.g., telomerase catalytic activity) are used, *inter alia*, to inhibit telomerase activity in a cell (e.g., by acting as "dominant negative mutants"). The hTRT variants and polynucleotides encoding them, as described herein, are similarly useful in screening assays for identifying agents that modulate telomerase activity.

The hTRT variants of the present invention are characterized by one or more deletions or mutations, relative to a naturally occurring hTRT polypeptide, in defined regions of the protein, as `

Processive telomerase catalytic activity can be assayed by a variety of methods, including the "conventional assay" (Morin, 1989, *Cell* 59:521), the TRAP assay (U.S. Patent No. 5,629,154; see also, PCT publication WO 97/15687, PCT publication WO 95/13381; Krupp et al. *Nucleic Acids Res.*, 1997, 25: 919; Wright et al., 1995, *Nucl. Acids Res.* 23:3794), the "dot blot immunoassay" (U.S. Patent Application Serial Number 08/833,377), and other assays (e.g., Tatematsu et al., 1996, *Oncogene* 13:2265). The TRAPeze™ Kit (Oncor,

Inc., Gaithersburg, MD) may be used. The telomerase substrate used in these assays may have a natural telomere sequence, or may be have a synthetic oligonucleotide with a different sequence (see, e.g., Morin, 1989, *Cell* 59:521; Morin, 1991, *Nature* 353:454-56).

As used herein, an hTRT variant is considered to have a specified activity if the activity is exhibited by either the hTRT variant polypeptide without an associated hTR RNA or in an hTRT-hTR complex. Each of the hTRT activities described *supra* is also described in detail in copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549.

II. hTRT Variants Described

a) hTRT Variants with Telomerase Catalytic Activity

It has now been demonstrated that large regions of the hTRT protein can be mutated (e.g., deleted) without loss of telomerase catalytic activity. Sites of mutation (e.g., deletion) are described herein with reference to the amino acid sequence provided in Figure 1 and encoded in plasmid pGRN121 (ATCC accession number 209016); however it will be recognized that the same or equivalent mutations may be made in other hTRT polypeptides, e.g., naturally occurring variants such as polymorphic variants, hTRT fusion proteins, hTRT homologs (e.g., from non-human species), and the like. For ease of discussion, the residues of the full-length hTRT protein having a sequence as provided in Figure 1 are referred to herein by number, with the amino-terminal methionine (M) in Figure 1 numbered "1", and the carboxy-terminal aspartic acid (D) numbered "1132".

Regions of the hTRT protein that can be mutated (e.g., deleted) without abolishing telomerase catalytic activity include the regions from amino acid residues 192 to 323 (inclusive) and residues 415 to 450 (inclusive). As is demonstrated in the experiments described *infra*, all or part of either of these regions, or all or part of both of them, can be deleted without abolishing the telomerase catalytic activity of the protein. The regions from amino acid residues 192 to 323 and residues 415 to 450 may be referred to as "nonessential" regions of hTRT (i.e., not essential for telomerase catalytic activity). Thus, in various embodiments, the hTRT variants of the invention comprise deletions of, or other mutations in, these nonessential regions of hTRT. As described in Section IV, *infra*, certain mutations (e.g., deletion of residues 415-450) alter RNA-binding characteristics of the hTRT variant.

Examples of mutations that can be made in the hTRT polypeptides of the invention include deletions, insertions, substitutions, and combination of mutations. Thus, in some embodiments the mutation is a deletion of at least one, typically at least about 10, and often at least about 25, at least about 50, or at least about 100 amino acid residues relative to a naturally occurring hTRT. In alternative embodiments, the mutation is a single amino acid substitution in a "non-essential" region, or a combinations of substitutions. Substitutions may be conservative substitutions or non-conservative substitutions. In still other embodiments, the mutation is an insertion or substitution of amino acids, for example the insertion of residues that encode an epitope tag or novel proteolytic site. Substitutions may be of one or more (e.g., all) of the residues in the above-mentioned regions or may be combined with deletions so that, e.g., a shorter heterologous sequence is a substituted for a longer hTRT sequence. It will be appreciated, as noted *supra*, that in some embodiments the hTRT variant has more than one different type of mutation relative to a naturally occurring hTRT protein (e.g., a deletion and a point mutation).

The hTRT variants of the invention have certain advantages compared to naturally occurring hTRT proteins. In some embodiments, mutations may confer more efficient *in vitro* expression of active hTRT (e.g., in expression systems in which shorter polypeptides are more efficiently expressed than longer polypeptides), may provide sequences that aid in purification (e.g., an epitope tag sequence), or may add a new functional moiety to the hTRT polypeptide (e.g., a 3'→5' exonuclease domain from DNA polymerase I).

As noted *supra*, the hTRT variant polypeptides of the invention comprising mutations (e.g., deletions) in the "non-essential" regions of the hTRT retain telomerase catalytic activity. These variants, and polynucleotides that encode them, are useful in any application for which other catalytically active hTRT proteins (e.g., wild-type hTRT proteins) or polynucleotides may be used, including, *inter alia*, in therapeutic, diagnostic, and screening uses. Exemplary uses of hTRT polypeptides and polynucleotides are described in additional detail in the afore cited copending applications (e.g., U.S.S.N. 08/912,951 and 08/974,549).

In one embodiment, the hTRT variant of the invention is used to increase the proliferative capacity of a cell by, e.g., increasing telomerase activity in the cell (see, Bodnar et al. *supra*, and copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549 for a detailed description of exemplary methods). Briefly, in one embodiment, a polynucleotide comprising (i) a sequence encoding the hTRT variant polypeptide; (ii) an operably linked promoter (e.g., a heterologous promoter); and, (iii) optionally polyadenylation and termination signals, enhancers, or other regulatory elements, is introduced into a target cell (e.g., by transfection, lipofection, electroporation, or any other suitable method) under conditions in which the hTRT variant polypeptide is expressed. The expression in the cell of the catalytically active hTRT variant of the invention results in increased proliferative capacity (e.g., an immortal phenotype).

In another embodiment, the hTRT variant is used for *in vitro* reconstitution (IVR) of a telomerase ribonucleoprotein (e.g., comprising the hTRT variant polypeptide and a template RNA, e.g., hTR) that has telomerase catalytic activity. *In vitro* reconstitution methods are described in, e.g., Weinrich et al., 1997, *Nat. Genet.* 17:498, and copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549. Briefly, in one embodiment, an expression vector encoding an hTRT variant of the invention is expressed in an *in vitro* expression system (e.g., a coupled transcription-translation reticulocyte lysate system such as that described in U.S. Patent No. 5,324,637). In a particular embodiment, the hTRT variant polypeptide is coexpressed with hTR. In an alternative embodiment, the hTRT variant and hTR are separately expressed and then combined (mixed) *in vitro*. In the latter method, the hTR RNA and/or hTRT polypeptide may be purified before mixing. In this context, the hTRT polypeptide is "purified" when it is separated from at least one other component of the *in vitro* expression system, and it may be purified to homogeneity as determined by standard methods (e.g., SDS-PAGE). The *in vitro* reconstituted (IVR) telomerase has a variety of uses; in particular it is useful for identifying agents that modulate hTRT activity (e.g., drug screening assays).

(b) Deletion Variants Lacking Telomerase Catalytic Activity

In an other aspect, the invention provides hTRT deletion variants that lack telomerase catalytic activity (i.e., having less than 1% of the wild type activity), as well as polynucleotides encoding the variants lacking telomerase catalytic activity. In particular, the invention provides variants comprising one or more of the following deletions relative to wild-type hTRT: residues 192-450, 637-660, 638-660, 748-766, 748-764, and 1055-1071. These variants are referred to herein as "PCA$^-$ variants" (processive telomerase catalytic activity minus variants).

The PCA$^-$ variant proteins and polynucleotides of the invention lacking telomerase catalytic activity are used in, *inter alia*, therapeutic, screening and other applications. For example, PCA– variants are useful as dominant negative mutants for inhibition of telomerase activity in a cell. In one embodiment, a PCA$^-$ variant is introduced into a cell (e.g., by transfection with a polynucleotide expression vector expressing the PCA$^-$ variant), resulting in sequestration of a cell component (e.g., hTR) required for accurate telomere elongation. Thus, for example, administration of a polypeptide that binds hTR, a DNA primer, a telomerase-associated protein, or other cell component, but which does not have telomerase catalytic activity, is used to reduce endogenous telomerase

activity in the cell or to otherwise interfere with telomere extension (e.g., by displacing active telomerase from telomeric DNA). Similarly, in certain embodiments, a PCA$^-$ variant of the invention having one or several hTRT activities (i.e., other than processive telomerase catalytic activity) is used for screening for agents that specifically modulate (inhibit or activate) a telomerase activity other than telomerase catalytic activity. The use of hTRT variants as dominant negative mutants, and in other applications, is described in detail in copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549.

III. Making hTRT Variants

The hTRT variant polypeptides and polynucleotides of the invention may be produced using any of a variety of techniques known in the art. In one embodiment, a polypeptide having the desired sequence, or a polynucleotide encoding the polypeptide, is chemically synthesized (see, e.g., Roberge, et al., 1995, *Science* 269:202; Brown et al., 1979, *Meth. Enzymol.* 68:109). More often, the hTRT variant polypeptides and polynucleotides of the invention are created by manipulation of a recombinant polynucleotide encoding an hTRT polypeptide. Examples of suitable recombinant polynucleotides include pGRN121, *supra*, and other hTRT cDNA and genomic sequences.

Methods for cloning and manipulation of hTRT encoding nucleic acids (e.g., site-specific mutagenesis, linker scanning mutagenesis, and the like) are well known in the art and are described, for example, in Sambrook et al., 1989, MOLECULAR CLONING: A LABORATORY MANUAL, 2ND ED., VOLS. 1-3, Cold Spring Harbor Laboratory, and Ausubel et al., 1997, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing and Wiley-Interscience, New York. One convenient method for producing a polynucleotide encoding a desired hTRT deletion variant is by restriction digestion and subsequent ligation of a hTRT polynucleotide, to remove a region(s) of the polynucleotide encoding the amino acid residues to be deleted. If desired, restriction sites can be introduced or removed from a synthetic or naturally occurring hTRT gene to facilitate the production and detection of variants.

Typically, the recombinant polynucleotide encoding an hTRT variant of the invention is linked to appropriate regulatory elements (e.g., promoters, enhancers, polyadenylation signals, and the like) and expressed in a cell free system (see, e.g., Weinrich et al., *supra*), in bacteria (e.g., *E. coli*), in *ex vivo* animal cell culture (see, e.g., Bodnar et al., *supra*), in animals or plants (e.g., transgenic organisms or in gene therapy applications), or by any other suitable method. Suitable expression systems are well known in the art and include those described in Weinrich et al., and Bodnar et al., both *supra*, and in e.g., copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549.

Additional hTRT variants of the invention may be made using "DNA shuffling" *in vitro* recombination technology (see, e.g., Crameri et al., 1998, *Nature* 391:288-291; Patten et al., 1997, *Curr. Opin. Biotechnol.* 8:724-733, Stemmer, 1994, *Nature* 370:389-391; Crameri et al., 1996, *Nature Medicine,* 2:1-3; Crameri et al., 1996, *Nature Biotechnology* 14:315-319; WO 95/22625; Stemmer, 1995, *Science* 270:1510; Stemmer et al., *1995, Gene,* 164, 49-53; Stemmer, 1995, *Bio/Technology,* 13:549-553; Stemmer, 1994, *Proc. Natl. Acad. Sci. USA* 91:10747-10751). The specific deletion variants described *supra,* "wild-type hTRT" and non-human hTRT-homologs may be used in individually or various combinations as starting substrates to produce novel polypeptides with the desired activity. The activity or activities of the resulting polypeptides determined using the assays described in Section I, *supra.*

IV. Exemplary hTRT Variants

a) Generally

Exemplary hTRT variants were created by *in vitro* mutagenesis of polynucleotides encoding a full-length hTRT protein using the method of Perez et al., 1994, *J. Biol. Chem.* 269:22485-87. The mutant polynucleotides were expressed *in vitro* and telomerase reconstituted by *in vitro* mixing of hTRT and human telomerase RNA as described in Weinrich et al., 1997, *supra*. Reconstitution reactions were carried out using 0.5 pmole, 20 pmole, and, in some cases, other amounts of added hTR. Telomerase processive catalytic activity was assayed using a modified TRAP assay (Weinrich et al., 1997, *supra*). The results are summarized in Table 1.

TABLE 1

Deletion Name	Oligo	Amino acids deleted	Activity[1]
pGRN234	RT1 + RT2	none (delete *Nco*I site)	+
pGRN226	RT3A	192-323	+
RT3	RT3	200-326	+
pGRN237	RT4A	192-271	+
RT4	RT4	200-271	+
pGRN210	LM122-Nuc	222-240	+
pGRN235	RT5	415-450	+
pGRN242	RT3A+RT5	192-326 + 415-450	+
pGRN243	RT4A+RT5	192-271 + 415-450	+
pGRN240	RT3A/5	192-450	-
pGRN238	RT6A	637-660	-
RT6	RT 6	638-660	-
pGRN239	RT8A	748-766	-
RT8	RT8	748-764	-
pGRN241	RT10	1055-1071	-
pGRN236	RT11	1084-1116	-
pGRN209	LM121-WG	930-934	-
pGRN231		560-565	-

"+" = at least 40% activity compared to *in vitro* reconsitution using wild-type hTRT (e.g., encoded by pGRN125; see Weinrich et al., 1997, *supra*)
"-" = less than 1% activity .

Certain of the hTRT variants described *supra* are altered in their ability to bind hTR. The variants encoded by pGRN235, pGRN242 and pGRN243 exhibited telomerase activity when 20 pmoles hTR (template RNA) was included in the reconstitution reaction, but showed a low or undetectable level of activity when 0.5 pmoles of hTR was used. The variable activity of these variants indicates that these variants have altered (e.g.,

decreased) hTR binding activity. Thus, the region from 415 to 450 is likely involved in RNA binding (e.g., by affecting the conformation of the protein).

This result suggests that the region immediately upstream of residue 415, corresponding to the conserved "CP" domain (Bryan et al., 1998, *Proc. Nat'l. Acad. Sci.* 95:8479-8484) is a region of contact between the hTRT protein and hTR (*e.g.*, corresponding to about residues 405 to 418 as set forth in Figure 1). This conclusion is supported by the relative lack of conservation of sequence when human and mouse TRT sequences are compared in the region corresponding to hTRT residues 415-450.

hTR binding to hTRT was also affected by mutations and deletions in the region 560-565. RNA binding was assayed by adding purified hTR to epitope tagged TRT proteins (*i.e.*, including a FLAG sequence; Immunex Corp, Seattle WA). The hTR and protein were incubated under conditions under which tagged "wild-type" hTRT associates with template RNA (hTR), and the hTRT protein or hTRT-hTR complex (if present) were immunoprecipitated. The precipitated complex was assayed for the presence and amount of associated RNA. Deletion of residues 560-565 dramatically decreased the binding of hTR by hTRT, with the concurrent expected decrease in telomerase activity (see Table 1, pGRN231). Mutation of phenylalanine (F) to alanine (A) mutation at position 561 of hTRT (the "F561A" variant; see, Weinrich et al., 1997, *supra*) resulted in reduced binding of hTR: this variant did not effectively bind hTR in association reactions when hTR was present at 0.5 pmoles, and showed less-than wild-type binding at 20 pmoles hTR. Mutation of tyrosine at 562 to alanine similarly resulted in a loss of hTR binding activity (e.g., about a 70-80% reduction compared to the wild-type sequence). Mutation of threonine at position 564 to alanine resulted in a decrease in RNA binding by approximately 20% compared to wild-type. In contrast, mutation of residues 560 (F) and 565 (E) to alanine did not affect hTR binding. These results indicate that the region from 560-565 is involved in RNA binding, e.g., by providing residues that contact hTR.

As will be apparent to one of skill advised of these results, the telomerase reconstitution may be inhibited using peptides comprising the sequence corresponding the hTRT residues 405-418, 560-565, or fragments thereof, or peptide mimetics of such sequences. Thus, in one embodiment of the present invention, telomerase activity in a cell or an *in vitro* composition in which TRT protein and TR RNA are present, such as a telomerase reconstitution assay, is reduced by introducing to the cell or *in vitro* composition a polypeptide comprising the sequence FFYVTE (SEQ. ID NO:3), a polypeptide comprising the sequence YGVLLKTHCPLRAA (SEQ. ID NO:4), a polypeptide consisting essentially of FFYVTE (SEQ. ID NO:3), a polypeptide consisting essentially of FYVT (SEQ. ID NO:5), a polypeptide consisting essentially of YGVLLKTHCPLRAA (SEQ. ID NO:4), a fragment of at least three residues of the aforementioned polypeptides, or a peptide analog or mimetic of the polypeptide of any of the aforementioned compositions.

Peptide mimetics (or peptide analogs) are well known and are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template polypeptide (Fauchere, 1986, *Adv. Drug Res.* 15:29; Veber et al., 1985, *TINS* p.392; and Evans et al., 1987, *J. Med. Chem.* 30:1229). Generally, peptidomimetics are structurally similar to the paradigm polypeptide having the sequence from hTRT but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, -CH'CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$, and $-CH_2SO-$. Peptide mimetics may have significant advantages over polypeptide embodiments of this invention, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others. In addition to modifications to the peptide backbone, synthetic or non-naturally occurring amino acids can also be used to substitute for the amino acids present in the polypeptide or in the functional moiety of fusion proteins. Synthetic or

non-naturally occurring amino acids refer to amino acids which do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein. Preferred synthetic amino acids are the d-α-amino acids of naturally occurring l-α-amino acid, mentioned above, as well as non-naturally occurring d- and l-α-amino acids represented by the formula H2NCHR5COOH where R5 is 1) a lower alkyl group, 2) a cycloalkyl group of from 3 to 7 carbon atoms, 3) a heterocycle of from 3 to 7 carbon atoms and 1 to 2 heteroatoms selected from the group consisting of oxygen, sulfur, and nitrogen, 4) an aromatic residue of from 6 to 10 carbon atoms optionally having from 1 to 3 substituents on the aromatic nucleus selected from the group consisting of hydroxyl, lower alkoxy, amino, and carboxyl, 5) -alkylene-Y where alkylene is an alkylene group of from 1 to 7 carbon atoms and Y is selected from the group consisting of (a) hydroxy, (b) amino, (c) cycloalkyl and cycloalkenyl of from 3 to 7 carbon atoms, (d) aryl of from 6 to 10 carbon atoms optionally having from 1 to 3 substituents on the aromatic nucleus selected from the group consisting of hydroxyl, lower alkoxy, amino and carboxyl, (e) heterocyclic of from 3 to 7 carbon atoms and 1 to 2 heteroatoms selected from the group consisting of oxygen, sulfur, and nitrogen, (f) -C(O)R2 where R2 is selected from the group consisting of hydrogen, hydroxy, lower alkyl, lower alkoxy, and -NR3R4 where R3 and R4 are independently selected from the group consisting of hydrogen and lower alkyl, (g) -S(O)nR6 where n is an integer from 1 to 2 and R6 is lower alkyl and with the proviso that R5 does not define a side chain of a naturally occurring amino acid. Other preferred synthetic amino acids include amino acids wherein the amino group is separated from the carboxyl group by more than one carbon atom such as β-alanine, γ-aminobutyric acid, and the like.

It will also be recognized by those of skill upon reviewing these results that the compositions (e.g., polypeptides and mimetics) described *supra* can be used to identify telomerase association and activity inhibitors other than the disclosed polypeptide and mimetics. These compositions may be used, for example, in rational drug design for *e.g.,* computer modeling of telomerase activity modulators (e.g., modulators that inhibit the association of TRT and TR or that catalyze the disassociation of the telomerase complex), as positive controls in screens for modulators of telomerase activity, or in competition assays with candidate telomerase activity modulators.

b) Methods

Mutagenesis of the hTRT coding sequence of pGRN125 was carried out using the methods described by Perez et al., 1994, *J. Biol. Chem.* 269:22485-87. Most of the deletion mutants were generated from the plasmid pGRN125 (Weinrich et al., 1997, *supra*). Deletion mutants pGRN235 and pGRN236 were made in a secondary round of mutagenesis in an altered pGRN234. pGRN234 was generated by mutating (deleting) the *Nco* I site in pGRN125 (changing CAC to CAT in the histidine residue at position 754) and introducing a new *Ncol* site at the translation start site (ATG). Table 2 shows exemplary oligonucleotides used to generate the plasmids expressing the deletion variants of the invention.

TABLE 2

Oligo Name	Oligo sequence 5'-3'	length	Description	SEQ. ID NO:
RT1	GAAGGCCGCCCACGGGCACGTCC GC	25	Mutagenesis oligo to delete Nco I site from pGRN125	6
RT2	CCCGGCCACCCCAGCCATGGCGC GCGCTCCCC		Mutagenesis oligo to create Nco I site @ ATG of pGRN 125	7
RT5	TACGGGGTGCTCCTCAAGACGCAC TGCCCGCTGCTCCGCCAGCACAGC AGCCCCTGGCAG	60	Mutagenesis oligo to create a deletion of aa 415-450 in pGRN125	8
RT10	TACTCCATCCTGAAAGCCAAGAAC GCAGGGCTGTGCCACCAAGCATTC CTGCTCAAGCTG	60	Mutagenesis oligo to create a deletion of aa 1055-1071 in pGRN125	9
RT11	CTGTGCCACCAAGCATTCCTGCTC AAGCTGGCCGCAGCCAACCCGGC ACTGCCCTCAGAC	60	Mutagenesis oligo to create a deletion of aa 1083-1116 in pGRN125. Oligo creates a NheI site.	10
RT3A	ACTCAGGCCCGGCCCCCGCCACA CGCTAGCGAGACCAAGCACTTCCT CTACTCCTCAGGC	60	Mutagenesis oligo to create a deletion of aa 192-323 in pGRN125. Oligo creates a NheI site.	11
RT4A	ACTCAGGCCCGGCCCCCGCCACA CGCTAGCGTGGTGTCACCTGCCAG ACCCGCCGAAGAA	60	Mutagenesis oligo to create a deletion of aa 192-271 in pGRN125. Oligo creates a NheI site.	12
RT6A	ATCCCCAAGCCTGACGGGCTGCGG CCGATTGTTAACATGCTGTTCAGCG TGCTCAACTACGAGCGGGCG	69	Mutagenesis oligo to create a deletion of aa 638-660 in pGRN125. Oligo creates a Hpa I site.	13
RT8A	ACGTACTGCGTGCGTCGGTATGCC GTGGTCACAGATCTCCAGCCGTAC ATGCGACAGTTCGTG	63	Mutagenesis oligo to create a deletion of aa 748-766 in pGRN125. Oligo creates a Bgl II site.	14
RT3A/5	ACTCAGGCCCGGCCCCCGCCACA CGCTAGCCTGCTCCGCCAGCACAG CAGCCCCTGGCAG	60	Mutagenesis oligo to create a deletion of aa 192-450 in pGRN125. Oligo creates a NheI site.	15
LM121-WG	GTTCAGATGCCGGCCCACGGCCTA TTCCCTCTAGATACCCGGACCCTG GAGGTGCAGAGCGAC	63	Mutagenesis oligo to delete aa 930-934. Oligo introduces a new XbaI site	16
LM122-Nuc	CCCTGGGCCTGCCAGCCCCGGGT GCCGGCGCTGCCCCTGAGCCGGA GCGG	50	Mutagenesis oligo to delete aa 222-240. Oligo introduces a new Nae I site	17
RT3	GCTAGTGGACCCCGAAGGCGTCTG GGATGCGAGACCAAGCACTTCCTC TACTCCTCAGGC	60	Mutagenesis oligo to create a deletion of aa200-323 in pGRN125	18
RT4	GCTAGTGGACCCCGAAGGCGTCTG GGATGCGTGGTGTCACCTGCCAGA CCCGCCGAAGAA	60	Mutagenesis oligo to create a deletion of aa 200-271 in pGRN125	19
RT6	GACGGGCTGCGGCCGATTGTGAAC ATGGACCTGTTCAGCGTGCTCAAC TACGAGCGGGCG	60	Mutagenesis oligo to create a deletion of aa 638-660 in pGRN125	20
RT8	ACGTACTGCGTGCGTCGGTATGCC GTGGTCACCTTGACAGACCTCCAG CCGTACATGCGA	60	Mutagenesis oligo to create a deletion of aa 748-764 in pGRN125	21

V. Definitions

The following terms are defined *infra* to provide additional guidance to one of skill in the practice of the invention:

As used herein, a polypeptide region in a first polypeptide "corresponds" to a region in a second polypeptide when the amino acid sequences of the two regions, or flanking the two regions, are the same or substantially identical. Sequences can be aligned by inspection (e.g., alignment of identical sequences) or by computer implemented alignment of the two sequences. Thus, for example, the residues 192 to 323 of the hTRT polypeptide having the sequence set forth in Figure 1 "correspond" to residues in the same position in a hTRT polypeptide that differs from the Figure 1 sequence due to polymorphic variation, or other mutations or deletions (e.g., when the two polypeptides are optimally aligned). Alignments may also be carried out using the GAP computer program, version 6.0 (Devereux et al, 1984, *Nucl. Acid. Res.* 12:387; available from the University of Wisconsin Genetics Computer Group, Madison, WI). The GAP program utilizes the alignment method of Needleham and Wunsch, 1970 *J. Mol. Biol.* 48: 443-453 as revised by Smith and Waterman, 1981, *Adv. Appl. Math* 2:482. The preferred default parameters for the GAP program include (1) the weighted comparison matrix of Gribskov and Burgess, 1986, *Nucl. Acid. Res.* 14:6745 as described by Schwartz and Dayhoff, eds., 1979, ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, National Biomedical Research Foundation, pp. 353-358 (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Alternatively, alignments can be carried out using the BLAST algorithm, which is described in Altschul et al., 1990, *J. Mol. Biol.* 215:403-410 using as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, 1989, *Proc. Natl. Acad. Sci. USA* 89:10915); alignments (B) of 50, expectation (E) of 10, M=5, and N=-4. A modification of BLAST, the "Gapped BLAST" allows gaps to be introduced into the alignments that are returned (Altschul et al., 1997, *Nucleic Acids Res* 1:3389-3402). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

When referring to an "activity" of an hTRT variant, a variant is considered to be active in an assay of it displays at least 40% of the activity characteristic of the hTRT polypeptide having the sequence set forth in Fig. 1 ("wild type"). A variant is considered to lack activity when it has less that 1% of the "wild type" activity. A variant with greater than 1% activity and less than 40% activity has "intermediate activity."

As used herein, "conservative substitution," refers to substitution of amino acids with other amino acids having similar properties (*e.g.*, acidic, basic, positively or negatively charged, polar or non-polar). The following six groups each contain amino acids that are conservative substitutions for one another: 1) alanine (A), serine (S), threonine (T); 2) aspartic acid (D), glutamic acid (E); 3) asparagine (N), glutamine (Q); 4) arginine (R), lysine (K); 5) isoleucine (I), leucine (L), methionine (M), valine (V); and 6) phenylalanine (F), tyrosine (Y), tryptophan (W) (see also, Creighton, 1984, PROTEINS, W. H. Freeman and Company).

All publications and patent documents cited in this application are incorporated by reference in their entirety and for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

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Geron Corporation

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Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser His
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Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly Pro
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35 40 45

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Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro Pro
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ccc gcc gcc ccc tcc ttc cgc cag gtg tcc tgc ctg aag gag ctg gtg 298
Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu Val
70 75 80

gcc cga gtg ctg cag agg ctg tgc gag cgc ggc gcg aag aac gtg ctg 346
Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val Leu
85 90 95

gcc ttc ggc ttc gcg ctg ctg gac ggg gcc cgc ggg ggc ccc ccc gag 394
Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro Glu
100 105 110

gcc ttc acc acc agc gtg cgc agc tac ctg ccc aac acg gtg acc gac 442

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Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr Asp
    115                 120                 125

gca ctg cgg ggg agc ggg gcg tgg ggg ctg ctg ctg cgc cgc gtg ggc   490
Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val Gly
130                 135                 140                 145

gac gac gtg ctg gtt cac ctg ctg gca cgc tgc gcg ctc ttt gtg ctg   538
Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val Leu
                150                 155                 160

gtg gct ccc agc tgc gcc tac cag gtg tgc ggg ccg ccg ctg tac cag   586
Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr Gln
            165                 170                 175

ctc ggc gct gcc act cag gcc cgg ccc ccg cca cac gct agt gga ccc   634
Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly Pro
        180                 185                 190

cga agg cgt ctg gga tgc gaa cgg gcc tgg aac cat agc gtc agg gag   682
Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg Glu
    195                 200                 205

gcc ggg gtc ccc ctg ggc ctg cca gcc ccg ggt gcg agg agg cgc ggg   730
Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg Gly
210                 215                 220                 225

ggc agt gcc agc cga agt ctg ccg ttg ccc aag agg ccc agg cgt ggc   778
Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg Gly
                230                 235                 240

gct gcc cct gag ccg gag cgg acg ccc gtt ggg cag ggg tcc tgg gcc   826
Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp Ala
            245                 250                 255

cac ccg ggc agg acg cgt gga ccg agt gac cgt ggt ttc tgt gtg gtg   874
His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val Val
        260                 265                 270

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Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala Leu
    275                 280                 285

tct ggc acg cgc cac tcc cac cca tcc gtg ggc cgc cag cac cac gcg   970
Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His Ala
290                 295                 300                 305

ggc ccc cca tcc aca tcg cgg cca cca cgt ccc tgg gac acg cct tgt  1018
Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro Cys
                310                 315                 320

ccc ccg gtg tac gcc gag acc aag cac ttc ctc tac tcc tca ggc gac  1066
Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly Asp
            325                 330                 335

aag gag cag ctg cgg ccc tcc ttc cta ctc agc tct ctg agg ccc agc  1114
Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro Ser
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ctg act ggc gct cgg agg ctc gtg gag acc atc ttt ctg ggt tcc agg  1162
Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser Arg
    355                 360                 365

ccc tgg atg cca ggg act ccc cgc agg ttg ccc cgc ctg ccc cag cgc  1210
Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln Arg
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                390                 395                 400

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Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg Ala
            405                 410                 415

gcg gtc acc cca gca gcc ggt gtc tgt gcc cgg gag aag ccc cag ggc  1354
Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln Gly
        420                 425                 430

tct gtg gcg gcc ccc gag gag gag gac aca gac ccc cgt cgc ctg gtg  1402
Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu Val
    435                 440                 445

cag ctg ctc cgc cag cac agc agc ccc tgg cag gtg tac ggc ttc gtg  1450
Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe Val
450                 455                 460                 465

cgg gcc tgc ctg cgc cgg ctg gtg ccc cca ggc ctc tgg ggc tcc agg  1498
Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser Arg
                470                 475                 480

cac aac gaa cgc cgc ttc ctc agg aac acc aag aag ttc atc tcc ctg  1546
His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser Leu
            485                 490                 495

ggg aag cat gcc aag ctc tcg ctg cag gag ctg acg tgg aag atg agc  1594
Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met Ser
        500                 505                 510

gtg cgg gac tgc gct tgg ctg cgc agg agc cca ggg gtt ggc tgt gtt  1642
Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys Val
    515                 520                 525

ccg gcc gca gag cac cgt ctg cgt gag gag atc ctg gcc aag ttc ctg  1690
Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe Leu
530                 535                 540                 545

cac tgg ctg atg agt gtg tac gtc gtc gag ctg ctc agg tct ttc ttt  1738
His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe Phe
                550                 555                 560

tat gtc acg gag acc acg ttt caa aag aac agg ctc ttt ttc tac cgg  1786
Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr Arg
            565                 570                 575

aag agt gtc tgg agc aag ttg caa agc att gga atc aga cag cac ttg  1834
Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His Leu
        580                 585                 590

aag agg gtg cag ctg cgg gag ctg tcg gaa gca gag gtc agg cag cat  1882
Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln His
    595                 600                 605

cgg gaa gcc agg ccc gcc ctg ctg acg tcc aga ctc cgc ttc atc ccc  1930
Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile Pro
610                 615                 620                 625

aag cct gac ggg ctg cgg ccg att gtg aac atg gac tac gtc gtg gga  1978
Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val Gly
                630                 635                 640

gcc aga acg ttc cgc aga gaa aag agg gcc gag cgt ctc acc tcg agg  2026
Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser Arg
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gtg aag gca ctg ttc agc gtg ctc aac tac gag cgg gcg cgg cgc ccc  2074
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Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg Ala
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Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro Glu
690                 695                 700                 705

ctg tac ttt gtc aag gtg gat gtg acg ggc gcg tac gac acc atc ccc   2218
Leu Tyr Phe Val Lys Val Asp Val Thr Gly Ala Tyr Asp Thr Ile Pro
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cag gac agg ctc acg gag gtc atc gcc agc atc atc aaa ccc cag aac   2266
Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln Asn
            725                 730                 735

acg tac tgc gtg cgt cgg tat gcc gtg gtc cag aag gcc gcc cat ggg   2314
Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His Gly
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cac gtc cgc aag gcc ttc aag agc cac gtc tct acc ttg aca gac ctc   2362
His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp Leu
    755                 760                 765

cag ccg tac atg cga cag ttc gtg gct cac ctg cag gag acc agc ccg   2410
Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser Pro
770                 775                 780                 785

ctg agg gat gcc gtc gtc atc gag cag agc tcc tcc ctg aat gag gcc   2458
Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu Ala
                790                 795                 800

agc agt ggc ctc ttc gac gtc ttc cta cgc ttc atg tgc cac cac gcc   2506
Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His Ala
            805                 810                 815

gtg cgc atc agg ggc aag tcc tac gtc cag tgc cag ggg atc ccg cag   2554
Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro Gln
        820                 825                 830

ggc tcc atc ctc tcc acg ctg ctc tgc agc ctg tgc tac ggc gac atg   2602
Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp Met
    835                 840                 845

gag aac aag ctg ttt gcg ggg att cgg cgg gac ggg ctg ctc ctg cgt   2650
Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu Arg
850                 855                 860                 865

ttg gtg gat gat ttc ttg ttg gtg aca cct cac ctc acc cac gcg aaa   2698
Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala Lys
                870                 875                 880

acc ttc ctc agg acc ctg gtc cga ggt gtc cct gag tat ggc tgc gtg   2746
Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys Val
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Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu Ala
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ctg ggt ggc acg gct ttt gtt cag atg ccg gcc cac ggc cta ttc ccc   2842
Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe Pro
    915                 920                 925

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Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe Asn
                950                 955                 960

cgc ggc ttc aag gct ggg agg aac atg cgt cgc aaa ctc ttt ggg gtc   2986
Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly Val
            965                 970                 975

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Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn Ser
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ctc cag acg gtg tgc acc aac atc tac aag atc ctc ctg ctg cag gcg   3082
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tgg aag aac ccc aca ttt ttc ctg cgc gtc atc tct gac acg gcc tcc   3178
Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala Ser
                1030                1035                1040

ctc tgc tac tcc atc ctg aaa gcc aag aac gca ggg atg tcg ctg ggg   3226
Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu Gly
            1045                1050                1055

gcc aag ggc gcc gcc ggc cct ctg ccc tcc gag gcc gtg cag tgg ctg   3274
Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp Leu
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tgc cac caa gca ttc ctg ctc aag ctg act cga cac cgt gtc acc tac   3322
Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr Tyr
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Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser Arg
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Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn Pro
                1110                1115                1120

gca ctg ccc tca gac ttc aag acc atc ctg gac tga tggccacccg        3464
Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
            1125                1130

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Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu
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Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr
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Gly Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val
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Gln Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly
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Glu Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg
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Gly Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg
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Gly Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp
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Ala His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val
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Val Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala
275 280 285

Leu Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His
290 295 300

Ala Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro

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Ser Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser
        355                 360                 365

Arg Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln
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Arg Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His
385                 390                 395                 400

Ala Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg
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Ala Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln
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Gly Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu
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Val Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe
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Val Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser
465                 470                 475                 480

Arg His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser
                485                 490                 495

Leu Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met
            500                 505                 510

Ser Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys
        515                 520                 525

Val Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe
    530                 535                 540

Leu His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe
545                 550                 555                 560

Phe Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr
                565                 570                 575

Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His
            580                 585                 590

Leu Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln
        595                 600                 605

His Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile
    610                 615                 620

Pro Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val
625                 630                 635                 640

Gly Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser
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Arg Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg
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Pro Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg
675 680 685

Ala Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro
690 695 700

Glu Leu Tyr Phe Val Lys Val Asp Val Thr Gly Ala Tyr Asp Thr Ile
705 710 715 720

Pro Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln
725 730 735

Asn Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His
740 745 750

Gly His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp
755 760 765

Leu Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser
770 775 780

Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu
785 790 795 800

Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His
805 810 815

Ala Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro
820 825 830

Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp
835 840 845

Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu
850 855 860

Arg Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala
865 870 875 880

Lys Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys
885 890 895

Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
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Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
915 920 925

Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
930 935 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945 950 955 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
965 970 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
980 985 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
995 1000 1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
1010 1015 1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
025 1030 1035 1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
1045 1050 1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
1060 1065 1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
1075 1080 1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
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Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
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Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
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